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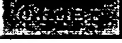
*Dev Biol* 1981 Apr 30;83(2):311-27

## **Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: an immunocytological study in the central nervous system.**

Sommer I, Schachner M

PMID: 6786942, UI: 81213186

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Other Formats:  Links: ☐ Order this document*Neurosci Lett* 1982 Apr 16;29(2):183-8

## Cell that are O4 antigen-positive and O1 antigen-negative differentiate into O1 antigen-positive oligodendrocytes.

Sommer I, Schachner M

In freshly dissociated viable cells of early postnatal mouse cerebellum, O1 antigen-positive oligodendrocytes have been eliminated by complement dependent immunocytolysis. Before seeding residual cells in culture, O4 antigen-positive cells were immunolabeled by O4 antibody which had been directly conjugated with rhodamine. After various periods of time in culture, cells were treated with fluorescein conjugated O1 antibody, in order to assay for the appearance of O1-positive oligodendrocytes. After 6.5 h in vitro, the first cells carrying both the previously applied rhodamine label and the freshly applied fluorescein label were seen. The simultaneous appearance of both labels indicates that O4-positive and O1-negative cells are direct precursors of O1 antigen-positive oligodendrocytes.

PMID: 6178060, UI: 82220724

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Other Formats:  Links: ☐ Order this document*Neurosci Lett* 1985 Mar 15;54(2-3):195-9

## Monoclonal cell surface antibodies do not produce short-term effects on electrical properties of mouse oligodendrocytes in culture.

Kettenmann H, Sommer I, Schachner M

Eleven monoclonal antibodies (O1-O11) directed against the surface of oligodendrocytes were applied individually or in combination during measurement of membrane potential, input resistance and K<sup>+</sup>-pump activity in explant cultures of mouse spinal cord. Antibody binding to oligodendrocytes was verified by indirect immunofluorescence. None of the antibodies affected the electrical properties studied. On the basis of these observations, it is possible to identify oligodendrocytes immunocytologically prior to electrophysiological characterization.

PMID: 2581183, UI: 85188876

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Other Formats: [Citation](#) [MEDLINE](#)Links: [Related Articles](#)☐ Order this document*Dev Biol* 1987 Sep;123(1):282-5

## The proteoglycan chondroitin sulfate is present in a subpopulation of cultured astrocytes and in their precursors.

Gallo V, Bertolotto A, Levi G

We have used an antibody raised against the bovine nasal cartilage proteoglycan chondroitin sulfate (CS) digested with chondroitinase ABC (anti-CS serum) to stain cerebellar glial cells maintained in culture. In cultures grown in the presence of serum, the antibody stained a subclass of GFAP+ astrocytes which we have previously shown to selectively bind the monoclonal antibodies A2B5 and LB1. Also the direct bipotential precursors of these cells, capable of differentiating into GFAP+ astrocytes or into Gal-C+, O1+ oligodendrocytes depending on the culture conditions, were stained, but stopped to produce CS when they differentiated into oligodendrocytes.

PMID: 3114027, UI: 87305195

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☐ Order this document*J Neurosci* 1987 Sep;7(9):2721-31

## Plasticity of developing cerebellar cells in vitro studied with antibodies against the NG2 antigen.

Levine JM, Stallcup WB

The NG2 antigen, a chondroitin-sulfate proteoglycan, is a cell surface marker for a class of smooth protoplasmic astrocytes found throughout the brain and at high frequency in the cerebellar molecular layer (Levine and Card, 1987). To study the development of the cerebellar astrocytes at the level of the single cell, we have analyzed the distribution of the NG2 antigen by indirect immunofluorescence in dissociated cell cultures prepared from postnatal cerebella and compared that distribution to the distribution of several other cell surface and intracellular antigens that identify specific cell types in cultures of nervous tissue. When cerebellar cells from 5 d rat pups were grown in a medium containing 10% fetal calf serum, the NG2-labeled cells, which constituted 0.1-1.0% of the total glial cells present, contained glial fibrillary acidic protein (GFAP)-immunoreactive filaments and bound monoclonal antibody A2B5, a surface marker for neurons and some astrocytes. Approximately 30% of the NG2-labeled cells were also labeled with tetanus toxin, an additional surface marker for neurons and immature astrocytes. Less than 2% of the cells were labeled with antibodies against galactocerebroside or with monoclonal antibody O1, both of which are surface markers for oligodendrocytes. About half the NG2-labeled cells exhibited high-affinity uptake of 3H-GABA, and this uptake was partially inhibited by both beta-alanine and DABA. Thus, the NG2 antigen is a cell surface marker for a subpopulation of the type II or fibrous astrocytes present in the cultures. When the cerebellar cells were grown in a chemically defined, serum-free medium, the NG2-labeled cells had a stellate morphology and between 50-60% of the cells bound tetanus toxin. Although almost all the cells bound antibody A2B5, less than 5% of the cells expressed either of the oligodendrocyte surface markers or GFAP immunoreactivity. As was the case with cells grown in serum-containing medium, 60% of the NG2-labeled cells had high-affinity uptake of 3H-GABA. However, this uptake was inhibited by DABA but not by beta-alanine. This phenotype may be the in vitro analog of the NG2-labeled, filament-lacking, smooth protoplasmic astrocytes identified in the intact adult cerebellum. The expression of these 2 phenotypes could be reversed by switching the tissue culture medium within 5 d of plating the cells. These results demonstrate that the in vitro environment can influence the phenotypic properties expressed by developing cerebellar astrocytes and suggest that smooth protoplasmic astrocytes may be developmentally related to glial cells of the O-2A lineage.

PMID: 3305799, UI: 87310618

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Other Formats: [Citation](#) [MEDLINE](#)Links: [Related Articles](#)☐ Order this document*Neurosci Lett* 1989 Jun 19;101(2):127-32

## Identification of astrocyte- and oligodendrocyte-like cells of goldfish optic nerves in culture.

Bastmeyer M, Beckmann M, Nona SM, Cronly-Dillon JR, Stuermer CA

Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Biologische Arbeitsgruppen, Tübingen, F.R.G.

Fish glial cells were obtained from cultivated segments of the optic nerve and raised in vitro. Two types of cells were identified as astrocyte- and oligodendrocyte-like glia by the monoclonal antibody Mab O1 (specific for oligodendrocytes) and the rabbit serum anti-goldfish glial fibrillary acidic protein (anti-G-GFAP). Cells of compact morphology were rare, and anti-G-GFAP positive and O1 negative. Multipolar cells in 5-day-old cultures were anti-G-GFAP but rarely O1 positive. In 5-week-old cultures, however, roughly 75% of the multipolar cells were double-labeled with both anti-G-GFAP and O1; 10% were single labeled with Mab O1 and 15% with anti-G-GFAP, respectively.

PMID: 2771160, UI: 89365784

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## Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids.

Bansal R, Pfeiffer SE

Department of Microbiology, University of Connecticut Medical School, Farmington 06032.

We have hypothesized that oligodendrocyte (OL) surface glycolipids, specifically galactocerebroside and sulfatide, play a role in the regulation of OL development by acting as sensors/transmitters of environment information. In support of this hypothesis we report here a reversible inhibition of OL progenitor cell differentiation by a monoclonal antibody [Ranscht mAb (R-mAb); Ranscht, B., Clapshaw, P. A. & Seifert, W. (1982) *Proc. Natl. Acad. Sci. USA* 79, 2709-2713] that reacts with these glycolipids. When isolated OL progenitors or mixed primary cultures are grown in the presence of the antibody, myelinogenic development is blocked in a dose-dependent manner at concentrations as low as 2 micrograms of IgG per ml. The inhibited cells express the OL progenitor markers O4 and vimentin but are negative for galactosylcerebroside, sulfatide, 2',3'-cyclic nucleotide 3'-phosphohydrolase, myelin basic protein, and myelin basic protein RNA expression. In contrast, the levels of total cellular protein and the expression of astrocytic glial fibrillary acidic protein in mixed cultures are not affected. Antibody-blocked cells have a distinctive morphology in which long, sparsely branched processes emanate from round cell bodies. Upon removing the perturbing antibody, the cells rapidly resume differentiation. Reverted mixed primary cultures, in which OL progenitors of several sequential developmental stages are present at the time of plating, differentiate more rapidly than control cultures, suggesting that the antibody-induced block results in a synchronization of developmental progression along the OL lineage by accumulating cells at the inhibition point. However, the normal temporal sequence of marker expression is maintained. Control studies with several other antibodies recognizing OL cell surface antigens, including HNK-1, neural cellular adhesion molecule (N-CAM), IA9, anticholesterol, and O1, did not inhibit development. Since the inhibition occurs in highly enriched populations of OL progenitors, the inhibition does not involve cell-cell interactions between OLs and other cell types but concerns interactions of OLs with themselves, soluble factors, or OL extracellular matrix molecules and adhesion factors that provide essential environmental signals required for normal myelinogenic development.

PMID: 2668957, UI: 89345627

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## Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development.

Bansal R, Warrington AE, Gard AL, Ranscht B, Pfeiffer SE

Department of Microbiology, University of Connecticut Health Center, Farmington.

Three monoclonal antibodies that react with antigens on the surface of developing oligodendrocytes in a stage-specific manner, O1, O4 (Sommer and Schachner, 1981), and R-mAb (Ranscht et al., 1982), have been studied with respect to their specificities for a number of purified lipids. The observed specificities were consistent regardless of how the antigens were presented to the antibodies. O1 reacted with galactocerebroside, monogalactosyl-diglyceride, and psychosine and, in addition, labeled an unidentified species in rat brain extracts. R-mAb reacted with galactocerebroside, monogalactosyl-diglyceride, sulfatide, seminolipid, and psychosine; the reaction of R-mAb with sulfatide was nearly equal to that with galactocerebroside. O4 reacted with sulfatide, seminolipid, and to some extent with cholesterol. However, oligodendrocyte progenitor cells labeling with O4 that had not yet begun to express the O1 antigen failed to incorporate  $^{35}\text{SO}_4$  or  $[^3\text{H}]$ galactose into sulfatide or seminolipid, the syntheses of which first appear in O1-positive cells. Therefore, O4 stains, in addition to sulfatide and seminolipid, and unidentified antigen that appears on the surface of oligodendrocyte progenitors prior to the expression of sulfatide and galactocerebroside. In primary cultures of rat brain, developing O4+ oligodendrocyte progenitors stained slightly earlier with R-mAb than with O1, and thus R-mAb transiently stained a larger population of oligodendrocytes than did O1. None of the three antibodies produced a detectable reaction on Western immunoblot after separation of brain proteins on reducing gels. In conclusion, the results show that O4, R-mAb, and O1 have multiple overlapping specificities, including previously unrecognized cross-reactions.

PMID: 2600978, UI: 90096198

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*J Neurosci Res* 1991 Jul;29(3):299-307

## Identification of a cDNA clone specific for the oligodendrocyte-derived repulsive extracellular matrix molecule J1-160/180.

Fuss B, Pott U, Fischer P, Schwab ME, Schachner M

Department of Neurobiology, Swiss Federal Institute of Technology, Honggerberg.

A cDNA clone specific for the oligodendrocyte-derived extracellular matrix glycoproteins J1-160/180 was obtained from a lambda ZAPII expression library using polyclonal antibodies generated against mouse J1-160. The library was constructed from poly(A)(+)-RNA isolated from O1 antigen-positive rat oligodendrocytes. The cDNA clone expressed a fusion protein that was recognized by the J1-160/180-specific monoclonal antibodies 596, 619, and 620, and, weakly, 597. The fusion protein was not recognized by polyclonal antibodies to mouse J1/tenascin. The cDNA clone with an insert of approximately 5.6 kb in size contained the nucleotide sequence coding for the amino acid sequence of the N-terminus of a tryptic peptide derived from mouse J1-160. The developmental and tissue distribution of the mRNA recognized by the cDNA clone is in agreement with the described expression of the J1-160/180 proteins.

PMID: 1717703, UI: 92015362

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## Novel stage in the oligodendrocyte lineage defined by reactivity of progenitors with R-mAb prior to O1 anti-galactocerebroside.

Bansal R, Pfeiffer SE

Department of Microbiology, University of Connecticut Medical School, Farmington 06030.

The developmentally regulated appearance of surface immuno-reactivity of prolignodendroblasts [oligodendrocyte progenitors reacting with monoclonal antibodies A007 and O4, but not anti-galactocerebroside (GalC), i.e., A007/O4+GalC-] to monoclonal antibodies R-mAb and O1 was studied both in culture and in vivo. In both cases staining with R-mAb shortly preceded that with O1; that is, a transient population of R-mAb+O1- cells was observed. R-mAb-O1+ cells were not detected. Differential staining with R-mAb and O1 was also noted at the subcellular level. In younger cultures in which R-mAb+ cells were first acquiring O1 immunoreactivity, many of these cells were stained by O1 only on the cell bodies and proximal portions of the processes, whereas in contrast R-mAb stained the whole cell, including the distal portions of the processes. Only in older, more mature R-mAb+ cells did O1 also stain the distal portions of processes. The expression of reactivity to R-mAb and O1 was compared to the proliferative capacity of the cells. Proliferation [assessed by bromodeoxyuridine (BrdU) incorporation] of both R-mAb+ and O1+ cells was negligible both in culture and in vivo. However, treatment of cells in culture with 10 ng/ml basic fibroblast growth factor resulted in an enhancement of proliferation of the R-mAb+ cells. Within the proliferating R-mAb+BrdU+ population, 80% of the cells were O1- (i.e., anti-galactocerebroside negative). These events occur during a critical period of development when A007/O4+ prolignodendroblasts begin to become post-mitotic and express surface galactocerebroside.

PMID: 1433382, UI: 93059491

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## Proliferation and differentiation of O4+ oligodendrocytes in postnatal rat cerebellum: analysis in unfixed tissue slices using anti-glycolipid antibodies.

Warrington AE, Pfeiffer SE

Department of Microbiology, University of Connecticut School of Medicine, Farmington 06030.

We report the study of the in vivo morphology, differentiation, and proliferation of oligodendrocytes (OLs) and their progenitors identified by the antiglycolipid antibodies O4, R-mAb, and O1 in postnatal rat cerebellum, using a novel immunocytochemical staining protocol which allows the analysis of the expression of OL-specific glycolipids in live, unfixed brain slices. An analysis of the individual cells identified in double label immunocytochemistry indicated that the order of antigen expression in OLs during in vivo development is, first, antigens recognized by O4, second, antigens recognized R-mAb, and third, antigens recognized by O1. This order of antigen expression is correlated with increasing morphological complexity and is a pattern mimicked in many culture systems. In vivo O4 identified 3 distinct stages of the OL lineage: (1) morphologically simple proligerodendrocyte antigen+ (POA+) R-mAb- blast cells localized at the leading edge of myelinogenesis; (2) morphologically more complex R-mAb+O1- cells; and (3) actively myelinating O1+ [i.e., galactocerebroside+ (GalC)] OLs residing within the white matter. Only the POA+R-mAb- cells incorporated BrdU in animals that were prelabeled 3 hr before immunocytochemistry. We have demonstrated in vivo the subdivision of pre-GalC+ OL progenitors into shorter, biologically noteworthy, stages of maturation. A spatial comparison of the cell populations identified by O4, R-mAb, and O1 demonstrated a progressive wave of OL maturation from the base of the cerebellum toward the folia. The data are consistent with the hypothesis that multiprocessed O4+GalC- progenitors are the most mature stage of the OL lineage with significant proliferative capacity and the first postmigratory stage in normal development.

PMID: 1453495, UI: 93085789

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## Microtubule-associated protein MAP1B expression precedes the morphological differentiation of oligodendrocytes.

Vouyiouklis DA, Brophy PJ

Department of Biological and Molecular Sciences, University of Stirling, Scotland, U.K.

The microtubule-associated protein MAP1B is believed to play an important role in the outgrowth of neurites from neurons (Tucker and Matus, *Dev Biol* 130: 423-434, 1988). We have investigated the possibility that MAP1B might participate in the formation of processes in cultured oligodendrocytes by an analysis of the expression of MAP1B during oligodendrocyte progenitor development. The appearance of the antigens recognized by the monoclonal antibodies A2B5, O4, and O1 which define distinct stages in the maturation of progenitors, was compared with the developmental expression of MAP1B. MAP1B is first detectable in O4+ preoligodendrocytes prior to the acquisition of galactocerebroside and immediately before they develop the complex process-bearing morphology characteristic of terminally differentiated myelin-forming oligodendrocytes in the CNS. In contrast, astrocytes have negligible amounts of MAP1B. These results demonstrate that the expression of MAP1B precedes the development of the mature oligodendrocyte phenotype and suggest that interactions between microtubules and MAP1B might have a role in the formation and stabilization of myelin-forming processes.

PMID: 8350387, UI: 93353572

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## Astrocytes and neurons regulate the expression of the neural recognition molecule janusin by cultured oligodendrocytes.

Jung M, Pesheva P, Schachner M, Trotter J

Department of Neurobiology, University of Heidelberg, Germany.

Janusin (formerly designated J1-160/180) is an extracellular matrix glycoprotein highly homologous to tenascin, consisting of two major molecular forms of 160 and 180 kD expressed by oligodendrocytes and in myelin. Janusin expression is upregulated during myelination and in the adult it remains expressed at lower levels. It is also present at the node of Ranvier, where myelin, axon, and astrocytic process are in close contact. To gain an understanding of the regulatory mechanisms which may underlie expression of janusin, the differentiation stage-dependent expression of janusin was studied in cultures enriched in mouse oligodendrocytes and their precursor cells. Expression of janusin by these cells was highest on both A2B5+ and O4+ O1- oligodendroglial precursor cells and a subset of myelin associated glycoprotein-positive (MAG+) oligodendrocytes. Hardly any of the more differentiated O1+ or O10+ oligodendrocytes expressed janusin. Expression of janusin was influenced by co-culture with astrocytes or neurons. Astrocytes or astrocytic-conditioned culture supernatants elevated the expression of janusin by the more differentiated oligodendrocytes (O1+ or MAG+ cells), while its expression by oligodendroglial precursor cells was relatively unchanged. Platelet-derived growth factor, but not basic fibroblast growth factor, also elevated the expression of janusin by O1+ or O10+ oligodendrocytes. In contrast, co-culture with neurons originating from dorsal root ganglia or spinal cord decreased the expression of cell-bound janusin by oligodendrocytes and their precursor cells. These observations indicate that expression of janusin on these cells in culture is susceptible to opposing regulatory influences from astrocytes and neurons. Such influences may modulate the temporal and spatial distribution of janusin in the developing and adult central nervous system.

PMID: 8294147, UI: 94124185

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Other Formats:  Links: ☐ Order this document*Int J Dev Neurosci* 1993 Dec;11(6):755-64

## Concurrent isolation and characterization of oligodendrocytes, microglia and astrocytes from adult human spinal cord.

Whittemore SR, Sanon HR, Wood PM

Miami Project, University of Miami School of Medicine, FL 33136.

A cellular preparation of highly enriched oligodendrocytes was obtained from adult human spinal cord by Percoll gradient centrifugation followed by either differential adhesion or fluorescence-activated cell sorting after immunostaining with an antibody against galactocerebroside (O1). The adherent and O1-negative cell fractions were > 96% microglia. The non-adherent and O1-positive fractions were > 96% positive for the oligodendrocyte markers O4 and O1, 0-2% positive for glial fibrillary acidic protein, and were devoid of neuronal or microglial markers. If the oligodendrocyte fraction was co-cultured with purified dissociated rat dorsal root ganglion neurons, the oligodendrocytes adhered to the axons and their numbers increased over a 4 week period. However, myelin sheaths were not produced around axons in these cultures. In contrast, if the oligodendrocyte cell fraction was grown alone in culture for > 3 weeks, the number of oligodendrocytes decreased and a layer of astrocytes developed underneath the oligodendrocytes. The oligodendrocytes could be eliminated from these cultures by subsequent passaging, thus producing cultures of pure astrocytes. The astrocytes accumulated both K<sup>+</sup> and glutamate with kinetic properties similar to those reported for rodent astrocytes. We suggest that these astrocytes arose in part from an O4/O1-positive precursor which did not initially express glial fibrillary acidic protein. These results define a relatively simple method by which highly enriched populations of oligodendrocytes, astrocytes and microglia can be obtained from adult human spinal cord.

PMID: 7907836, UI: 94182475

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## Biochemical and cellular properties of three immortalized Schwann cell lines expressing different levels of the myelin-associated glycoprotein.

Toda K, Small JA, Goda S, Quarles RH

Myelin and Brain Development Section. National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland.

Biochemical and cellular properties of three immortalized Schwann cell lines expressing different levels of the myelin-associated glycoprotein (MAG) were compared. The S16 line generated by repetitive passaging was described previously and expresses a level of MAG comparable to that in adult sciatic nerve. The S42 line was generated independently by the same procedure, divides more slowly than the S16 line, and expresses an even higher level of MAG. The S16Y line arose spontaneously from a passage of the S16 cells, divides much more rapidly, and does not express MAG. The levels of MAG expression in the three lines are inversely related to their rates of proliferation, and MAG mRNA levels parallel the amounts of MAG. The S16 and S42 lines consist mainly of flat cells at low density and develop many processes at high density, whereas most of the S16Y cells are spindle-shaped, resembling primary Schwann cells in appearance. Surface immunostaining with the O4 antibody was positive for the S16 and S42 cells and negative for the S16Y cells, but all three lines were negative for surface staining with the O1 antibody. The overall protein compositions of the three lines are very similar, but the S16 and S42 cells express larger amounts of several glycoproteins than the S16Y cells, including the adhesion proteins, neural cell adhesion molecule, L1, and laminin. S16 and S42 cells (but not S16Y cells) also express P0 glycoprotein, galactocerebroside, and sulfatide, but, unlike MAG, these other myelin-related components were present at much lower levels than in adult nerve. Myelin basic protein and proteolipid protein were not detected in any of the lines, although all three lines contained proteolipid protein mRNA. 2',3'-Cyclic nucleotide 3'-phosphodiesterase and glial fibrillary acidic protein were present in all three lines. Conditions have not yet been found in which any of the lines will myelinate dorsal root ganglion neurons in vitro, but the S16 and S42 cells differ from the S16Y cells by clustering around neurons after 1 week in coculture. In many respects, the S16 and S42 cells biochemically resemble Schwann cells at an early stage in their preparation to myelinate and should be useful for investigating the cell biology of MAG and other myelin-related components.

PMID: 7523597, UI: 95016705

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Other Formats: **Citation** **MEDLINE**Links: **Related Articles**☐ Order this document*Glia* 1994 Nov;12(3):173-9**Regulation of gene expression in mature oligodendrocytes by the specialized myelin-like membrane environment: antibody perturbation in culture with the monoclonal antibody R-mAb.****Bansal R, Pfeiffer SE**

Department of Microbiology, University of Connecticut Medical School, Farmington 06030.

We have previously shown that the growth of oligodendrocyte progenitors in the presence of a monoclonal antibody (R-mAb) reacting with a cell surface component reversibly blocks their further differentiation at a specific, late progenitor stage of the lineage. This block is characterized by a nearly complete elimination of the onset of terminal differentiation at the level of RNA expression. In the present study, mature oligodendrocytes already expressing markers of terminal differentiation were exposed to R-mAb. This resulted in a retraction of cell processes and the formation of round, swollen cells, and a dose-dependent, antibody-specific partial reduction (30-50%) in the steady state levels of markers of terminal differentiation. Upon removing the perturbing antibody, all markers returned to control levels within 2 days. This inhibition was due to modulations of the levels of the specific mRNAs and proteins, not to cell loss. Total protein and levels of a marker of astrocytic differentiation were not affected by the treatment. Monoclonal antibody OI did not cause the effects observed with R-mAb. We conclude that the response of terminally differentiating oligodendrocytes to the effects of R-mAb is different from that of oligodendrocyte late progenitors. Whereas the latter appears to operate through perturbation of the onset of gene expression (mRNA transcription and/or stability), the partial down-regulation of previously activated myelinogenic gene expression appears to be due to the loss of a normal, myelin-like, membrane environment needed for the stability of myelin mRNA and protein components.

PMID: 7851986, UI: 95154930

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*Glia* 1995 Feb;13(2):130-40

## Elevated intracellular levels of cAMP induce olfactory ensheathing cells to express GAL-C and GFAP but not MBP.

Doucette R, Devon R

Department of Anatomy, College of Medicine, University of Saskatchewan, Saskatoon, Canada.

The primary olfactory pathway contains non-myelinating glial cells, called ensheathing cells, that exhibit a variety of phenotypes depending on their immediate environment. In vivo, these cells normally possess a mixture of astrocyte- and Schwann cell-specific phenotypic features. When co-cultured with dorsal root ganglion neurons, their phenotype can become more like that of a myelinating Schwann cell. The objective of this study was to determine whether ensheathing cells would express a myelinating phenotype in culture in the absence of neurons but in the presence of cAMP analogues that are known to induce the expression of myelin associated molecules in Schwann cell cultures. The ensheathing cell cultures were initiated using the nerve fiber layers of Theiler stage 23 rat olfactory bulb primordia and were fed for 1 day to 3 weeks with serum containing (1% or 10% FBS) or serum-free media to which was added different concentrations of dBcAMP (0.1 to 1 mM) or forskolin (10 microM). These cultures were double-labelled with a rabbit polyclonal antibody to S100 in combination with mouse anti-GAL-C (O1 and BRD1 hybridomas) or anti-MBP monoclonal antibodies. The remaining cultures were double-labeled with a rabbit polyclonal antibody to GFAP in combination with the BRD1 antibody. Treatment with dBcAMP or forskolin failed to induce ensheathing cells to express MBP regardless of the concentration. On the other hand, the treatment induced approximately one tenth of the cells to express GAL-C, and virtually all of the cells to express GFAP. These results indicate that although ensheathing cells can synthesize myelin associated molecules, the cAMP second messenger system appears to play a lesser role in controlling the expression of a myelinating phenotype in ensheathing cells than it does in Schwann cells.

PMID: 7544324, UI: 95377808

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*Ann Neurol* 1995 Jul;38(1):92-101

## Oligodendroglial development in human fetal cerebrum.

Rivkin MJ, Flax J, Mozell R, Osathanondh R, Volpe JJ, Villa-Komaroff L

Department of Neurology, Children's Hospital, Harvard Medical School, Boston, MA 02115, USA.

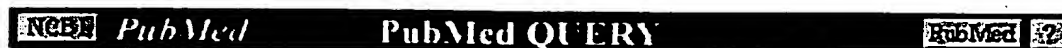
We have successfully established mixed glial cell primary cultures prepared from individual fetal human brains (15-18 weeks' gestation in age). Cultures were maintained for as long as 3 months in either 10% fetal calf serum (FCS) or serum-free chemically defined medium (CDM). By morphological and immunohistochemical criteria, the precursor cell for human oligodendrocytes (O-2A cell) was identified. This cell exhibited the bipolar morphology and A2B5-positive (A2B5+) immunoreactivity typical of the O-2A precursor cell. With time in culture, cells possessing a stellate morphology appeared, some of which stained with the O4 antibody, indicative of cell differentiation in the oligodendroglial lineage. At yet older culture age, arborized cells bearing the O1 (galactocerebroside, GC) immunohistochemical marker and displaying the morphological characteristics typical of more mature oligodendrocytes were found, confirming their oligodendroglial identity. Oligodendroglial differentiation was supported best by CDM rather than FCS. To complement these observations, double immunofluorescent studies were performed on parietal sections from human fetal brains at 20 to 22 weeks of gestation. Bipolar A2B5+, multipolar A2B5+/O4+, and arborized A2B5-/O1+ cells were found, thus confirming the presence of oligodendrocytes in human fetal brain at this stage of prenatal development and consistent with the observations made in cell culture.

### Comments:

- Comment in: *Ann Neurol* 1996 May;39(5):684-5

PMID: 7611731, UI: 95336158

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*Glia* 1995 Oct;15(2):95-104

## O-2A progenitors of the mouse optic nerve exhibit a developmental pattern of antigen expression different from the rat.

Fanarraga ML, Sommer I, Griffiths IR

Applied Neurobiology Group, University of Glasgow, Scotland.

In a previous study we demonstrated that differentiation and development of mouse oligodendrocytes is similar to that of the rat after the stage at which O4 is acquired. In this present study we compare directly the early differentiation of oligodendrocytes in the mouse and rat post natal optic nerve and show that the two species differ at the O-2A progenitor and prolodendroblast stages. Mouse progenitors show a variety of morphologies compared to the typical bipolar appearance in the rat. Many murine cells fail to immunolabel with A2B5, GD3, O4, and RmAb, classical markers for rat progenitors, prolodendroblasts, and immature oligodendrocytes. We find that these "unlabeled" cells stain for GAP-43 and that expression of GAP-43 overlaps A2B5 and GD3 in the earlier progenitors and O4, RmAb, and O1 in the later prolodendroblasts and immature oligodendrocytes. Our data suggest that in the development of the mouse O-2A progenitor cells there is a developmental discontinuity between the earlier markers such as A2B5 and GD3 and the later marker O4, which can be filled by GAP-43. We therefore consider that GAP-43 could be used in the mouse, in addition to the classical O-2A markers, for the study of the early oligodendrocyte lineage as it labels an otherwise undetectable O-2A population.

PMID: 8567073, UI: 96129322

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## Characterization of a novel oligodendrocyte cell surface molecule.

Zhou L, Szigeti V, Miller RH

Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA.

Cell-type-specific reagents have proven useful in the analysis of central nervous system (CNS) development and function. Most markers of oligodendrocytes are components of myelin sheath, which in the CNS is the specific product of oligodendrocytes. We have isolated a novel monoclonal antibody termed 2B10 which was raised against embryonic rat spinal cord tissue. In adult rat cerebrum, cerebellum, and spinal cord, 2B10 immunoreactivity is predominantly localized in white matter. 2B10 immunoreactivity is absent from peripheral nerve, suggesting that in the nervous system the 2B10 antigen is restricted to the CNS. Dissociated cell culture studies indicate that 2B10 labels a cell surface molecule, and its cellular distribution is coincident with O1 and myelin basic protein-positive oligodendrocytes. By contrast, 2B10 does not label GFAP-positive astrocytes. These data suggest that in the CNS the 2B10 antigen is expressed specifically on oligodendrocytes. Biochemical analysis indicates that 2B10 recognizes a protein with an apparent molecular weight of approximately 79,000 in reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Developmentally, 2B10 immunoreactivity is detectable in rat spinal cord at as early as embryonic day 14. The relative abundance of this molecule decreases during myelination, but is maintained at a sustained level throughout adulthood. The 2B10 antigen appears not to be a myelin-associated protein since it is not detected in purified myelin fractions. 2B10 immunoreactivity is not detectable in extracts of rat sciatic nerve, heart, kidney, muscle, and skin, but is detectable in extracts of spleen and thymus. These data suggest that the 2B10 antigen is a novel cell surface molecule that is expressed on oligodendrocyte lineage cells throughout development.

PMID: 8568937, UI: 96155758

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Other Formats: **Citation** **MEDLINE**Links: **Related Articles**☐ Order this document*J Neurocytol* 1995 Nov;24(11):818-24**Paranodal structural abnormalities in rat CNS myelin developing in vivo in the presence of implanted O1 hybridoma cells.**

Rosenbluth J, Liang WL, Liu Z, Guo D, Schiff R

Department of Physiology and Neuroscience, NYU School of Medicine, NY 10016, USA.

O1 hybridoma cells, which produce a monoclonal IgM antigalactocerebroside, were implanted into the spinal cords of immature and mature rats and the cords examined 5-24 days later. Study of the younger group, in which myelin was developing at the time of implantation, revealed examples of abnormal myelin sheaths in which the repeat period was markedly increased. The paranodal regions of these abnormal sheaths were superficially normal in configuration; i.e. myelin lamellae terminated one by one as 'terminal loops' that indented the axolemma and formed normal axoglial junctions displaying periodic 'transverse bands'. Neighbouring terminal loops are normally joined by tight junctions that block passage of tracers from the paranodal periaxonal space into the compact myelin, as seen after implantation of a control hybridoma. In the abnormal sheaths that developed after O1 implantation, in contrast, terminal loops were usually widely separated from each other. As a result, multiple pathways from the paranodal periaxonal space into the myelin sheath remained patent, forming potential routes for shunting nodal action currents. This subtle abnormality could thus compromise conduction, even though the sheaths might appear to be normally myelinated at the histological level. Equivalent abnormalities in human neurological diseases, including multiple sclerosis and paraproteinemic neuropathies, could underlie functional loss in the absence of frank demyelination.

PMID: 8576711, UI: 96163981

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## Oligodendrocyte-reactive O1, O4, and HNK-1 monoclonal antibodies are encoded by germline immunoglobulin genes.

Asakura K, Miller DJ, Pogulis RJ, Pease LR, Rodriguez M

Department of Neurology, Mayo Clinic and Foundation, Rochester, MN 55905, USA.

Natural or physiologic autoantibodies are present normally in serum, are polyreactive, are frequently of the IgM subtype, and are encoded by unmutated germline genes. We tested whether the oligodendrocyte-reactive O1, O4, A2B5, and HNK-1 IgM kappa monoclonal antibodies are natural autoantibodies by sequencing immunoglobulin (Ig) cDNAs and comparing these with published germline sequences. O1 VH was identical with unrearranged VH segment transcript A1 and A4. O4 VH had three and HNK-1 VH had six nucleotide differences from germline VH101 in the VH coding region. The D segment of O1 was derived from germline SP2 gene family. The D segments of O4 and HNK-1 were derived from DFL16 gene family. O4 JH and HNK-1 JH were encoded by unmutated germline JH4, whereas O1 JH was encoded by germline JH1 with one silent nucleotide change. O1 and O4 light chains were identical with myeloma MOPC21 except for one silent nucleotide change. HNK-1 V kappa was identical with germline V kappa 41 except for two silent nucleotide changes. O1 J kappa, O4 J kappa and HNK J kappa were encoded by unmutated germline J kappa 2. In contrast, A2B5 VH showed seven nucleotide differences from germline V1, whereas no germline sequence encoding A2B5 V kappa was identified. O1 and O4, but not A2B5 were polyreactive against multiple antigens by direct ELISA. Therefore, O1, O4 and HNK-1 Igs are encoded by germline genes, and have the genotype and phenotype of natural autoantibodies.

PMID: 8750831, UI: 96363014

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## Progressive remodeling of the oligodendrocyte process arbor during myelinogenesis.

Hardy RJ, Friedrich VL Jr

Brookdale Center for Molecular Biology, Mount Sinai Medical School, New York, NY 10029, USA.

Myelin sheaths develop in the central nervous system (CNS) as elaborations of the processes of oligodendrocytes. Although many details of the spiral wrapping of oligodendrocyte processes around axons and their subsequent transformation into myelin sheaths are known from thin-section electron-microscopic studies, the three-dimensional architecture of the myelin-forming cells is incompletely understood. To characterize this aspect of oligodendrocyte development, we labeled thick (100- to 300-microns) sections of developing murine CNS with oligodendrocyte marker antibodies, recorded individual cells in serial optical sections by confocal microscopy, and created whole-cell reconstructions of oligodendrocytes before and during the initiation of myelination. We distinguish three stages in the maturation of oligodendrocytes, which at all three stages are labeled by the O4, O1 and Ranscht monoclonals and by antibodies against the myelin-specific proteins CNP and myelin basic protein. Premyelinating oligodendrocytes, present before axonal ensheathment begins, emit multiple irregular processes which have predominant radial orientation. These processes, which generally terminate within 50 microns of the cell body, have a surface area 3-8 times or more that of the cell body itself and may represent a mechanism for sampling the local environment of each cell and for identifying target axons. Transitional cells have initiated one or more myelin sheaths; these cells progressively reduce the number of their radial processes as they increase the number of their myelin internodes. The radial processes of each transitional cell are most reduced in parts of the process arbor where ensheathment has begun, suggesting directional control in the elaboration or stability of the radial processes. Mature myelin-bearing oligodendrocytes entirely lack the radial processes and instead emit a few sparsely branching processes which connect cell bodies with myelin internodes. Three-dimensional analysis of the earliest stages in myelin sheath formation reveals two distinct phases. The initiating event in the formation of myelin internodes is the growth of thin unbranched processes, termed 'initiator processes', along axons. The second phase, spiral ensheathment of target axons, begins through the elaboration from each initiator process of lamellar extensions which extend circumferentially around the target axon and thereby form the first turn of its myelin sheath.

PMID: 8911764, UI: 97068487

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*J Neurosci* 1996 Apr 15;16(8):2635-41.

## Expanded CNS myelin sheaths formed in situ in the presence of an IgM antigalactocerebroside-producing hybridoma.

Rosenbluth J, Liang WL, Liu Z, Guo D, Schiff R

Department of Physiology and Neuroscience and Institute of Rehabilitation Medicine, New York University School of Medicine, New York 10016, USA.

When O1 hybridoma cells, which produce an IgM antigalacto-cerebroside, are implanted into the dorsal columns of 4-8 d rat spinal cord, some of the myelin that subsequently develops in the immediate vicinity displays an abnormal periodicity. The spacings that are seen cluster at approximately 19 nm and 31 nm, roughly two and three times the normal 11 nm spacing. In the expanded sheaths, major dense lines are separated by broad extracellular spaces containing a dense material in which single or double rows of approximately 10 nm circular profiles can be identified, consistent with the "central rings" of IgM molecules. Because IgM is multivalent, it may serve to link adjacent lamellae together in place of intrinsic myelin molecules that normally interact at close range. Extensive direct contact between myelin components of successive myelin lamellae is thus not essential to signal the growth of the oligodendrocyte membrane or the spiral wrapping of that membrane around axons during myelinogenesis, or to stabilize the myelin spiral that forms.

PMID: 8786439, UI: 96256699

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**Related Articles****Development**☐ Order this document*Development* 1996 Jul;122(7):2059-69

## **Oligodendrocyte progenitors are generated throughout the embryonic mouse brain, but differentiate in restricted foci.**

**Hardy RJ, Friedrich VL Jr**Brookdale Center for Molecular Biology, Mount Sinai Medical School, NY 10029, USA.  
hardy@anton.molbio.mssm.edu

Recent evidence from studies mapping the expression of putative oligodendrocyte progenitor specific mRNAs has suggested that oligodendrocyte progenitors arise during embryogenesis, in specific foci of the neuroectoderm. In order to test this hypothesis, we have assayed different regions of the embryonic central nervous system for their ability to generate oligodendrocytes following transplantation into neonatal cerebrum. To allow identification of donor-derived oligodendrocytes in wild-type host brain, we used the MbetaP transgenic mouse, which expresses lacZ in oligodendrocytes, as donor tissue. We found that tissue fragments derived from several levels of the anterior-posterior axis of the neural tube at E14.5 and E12.5, chosen to include (hindbrain, cervical and lumbar spinal cord), or exclude (dorsal telencephalon) putative foci of oligodendrocyte progenitors, all produced oligodendrocytes following transplantation. In addition, these same regions taken from E10.5, prior to the appearance of putative oligodendrocyte progenitor markers, also all yielded oligodendrocytes on transplantation. This indicates that precursor cells that can generate oligodendrocytes are widespread throughout the neuroectoderm as early as E10.5. We have also used the oligodendrocyte lineage-specific glycolipid antibodies O4, R-mAb and O1 to identify those regions of the developing brain that first support the differentiation of oligodendrocytes from their progenitor cells. We found that the first oligodendrocytes arise in prenatal brain at E14.5, in a restricted zone adjacent to the midline of the medulla. These cells are mitotically inactive, differentiated oligodendrocytes and, using light and electron microscopy, we show that they become functional, myelin-bearing oligodendrocytes. We have mapped the subsequent appearance of differentiated oligodendrocytes in the prenatal brain and show that they appear in a restricted, tract-specific manner. Our results suggest that oligodendrocytes are generated from neuroectodermal cells positioned throughout the rostrocaudal axis of the neural tube, rather than at restricted locations of the neuroectoderm. By contrast, the differentiation of such cells into oligodendrocytes does occur in a restricted manner, consistent with local regulation of oligodendrocyte progenitor differentiation.

PMID: 8681787, UI: 96281650

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*Eur J Neurosci* 1997 Oct;9(10):2213-7

## Characterization of a putative novel type of oligodendrocyte in cultures from rat spinal cord.

Fanarraga ML, Milward EA

Departamento de Biología Molecular, Universidad de Cantabria, Cardenal Herrera Oria s/n Santander, Spain.

Oligodendrocytes originate in different neural tube domains, within boundaries of expression of a series of patterning genes which condition the diverse morphogenetic programme of each area. Although neuronal and astrocyte heterogeneity are widely accepted, and despite accumulating evidence for oligodendrocyte heterogeneity in vivo, oligodendrocytes in vitro are currently considered as a homogeneous cell population. The present investigation demonstrates that oligodendrocyte diversity can be detected in vitro and characterizes a novel morphological class of O4-positive oligodendrocyte which is consistently identifiable in rat central nervous system cultures. These cells have a very characteristic epithelioid, unbranched and often lobulated morphology which enables their identification within 2 h of plating. Immunostaining shows that this morphological type is sometimes positive for GD3, A2B5 and vimentin, and most of the time positive for Ranscht antibody, O1 and Rip but negative for glial fibrillary acidic protein, OX-42, neuron-specific enolase, nestin and erbB2. The apparent levels and/or distributions of (i) microtubules, (ii) surface glycolipids recognized by O4, O1 and Ranscht antibody, and (iii) the less specific marker carbonic anhydrase II, typically differ from those of nearby classical, branched oligodendrocytes. Cells with this epithelioid morphology also express myelin basic protein and O10 (a proteolipid protein epitope), both of which are markers for mature oligodendrocytes. Conversely, O4+/O1- cells with this membranous appearance were also seen. Although these atypical oligodendrocytes were most abundant in spinal cord cultures (representing >10% of the O4+ population), they were not exclusive to this region and occurred at a low frequency in neonatal optic nerve cultures.

PMID: 9421182, UI: 98081535

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*J Neurosci Res* 1998 Jun 1;52(5):559-68

## Intracellular sulfatide expression in a subpopulation of astrocytes in primary cultures.

Berntson Z, Hansson E, Ronnback L, Fredman P

Institute of Clinical Neuroscience, Department of Psychiatry and Neurochemistry, Goteborg University, Molndal, Sweden. zarah.berntson@ms.se

A highly specific antibody against sulfatide, a myelin-associated glycolipid, has been investigated using indirect double immunocytochemistry in rat primary astroglial cultures from cerebral cortex. Sulfatide was expressed in a selected subpopulation of astrocytes (2-3%) and was found to be exclusively located intracellularly. The sulfatide-positive cells appeared in two different morphologies: flat and stellate. Immunolabeling of the astroglial cultures showed that sulfatide always co-existed with GFAP or S-100, and in some cells with GD3 (flat 90% and stellate 50%) or A2B5 (1%) antibody. The sulfatide-positive cells did not bind the O1 antibody, which is used as a marker for oligodendrocytes. Glial cultures from other regions and mixed cultures, with both neurons and glial cells, were examined and showed similar results. Biochemical analysis by TLC-ELISA verified the presence of sulfatide in the astroglial culture and showed decreasing amounts of sulfatide with days in vitro; 0.05 nmol/mg protein at day 10 and 0.01 nmol/mg protein at day 17. This analysis also showed that neither sulpholactosylceramide nor seminolipid was present, each of which also has affinity for the sulfatide antibody. This selective and intracellular expression encourages further identification of the astrocytes expressing sulfatide and the biological role of sulfatide in these cells.

PMID: 9632312, UI: 98294101

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## Developmental appearance of oligodendrocytes in the embryonic chick retina.

Ono K, Tsumori T, Kishi T, Yokota S, Yasui Y

Department of Anatomy, Shimane Medical University, Izumo, Japan. katsono@shimane-med.ac.jp

The axons of the optic nerve layer are known to be myelinated by oligodendrocytes in the chick retina. The development of the retinal oligodendrocytes has been studied immunohistochemically with antibodies against oligodendrocyte lineage: monoclonal antibodies O4 and O1, and an antibody against myelin basic protein. O4 positive (O4+) cells were first detected in the retina on the tenth day of incubation (embryonic day (E)10, stage 36). The labeled cells were located in the optic nerve layer close to the optic fissure. Most were unipolar in shape, extending a leading process with a growth cone toward the periphery of the retina. By E12, unipolar O4+ cells had spread to the middle of the retina. Many O4+ cells close to the optic fissure showed radial arrangement with extension of processes toward the inner limiting membrane. O1+ oligodendrocytes were first observed in the E14 retina positioned just above (interiorly to) retinal ganglion cells. These labeled cells extended fine processes in the optic nerve layer. Limited numbers of myelin basic protein-positive cells were present by E16 and located interiorly to the retinal ganglion cells. In addition to the oligodendrocyte in the optic nerve layer, a limited number of O4+ cells were observed in the inner nuclear layer by E14, and they became O1+ by E18. Furthermore, explant culture experiments showed E10 to be the youngest stage at which the retina contained oligodendrocyte precursors. An intravitreal injection of fluorescent dye 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) at E6 yielded O4+/DiI+ cells in the retina at E10, which provided direct evidence to support migration of oligodendrocyte precursor into the retina. The present results demonstrated the sequential appearance of the cells of oligodendrocyte lineage and the detailed morphology of the developing oligodendrocytes in the retina. These morphologic features strongly suggested that retinal oligodendrocytes were derived from the optic nerve and spread by migration through the optic nerve layer.

PMID: 9714145, UI: 98377947

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J. Neurosci.

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## Targeting of IgMkappa antibodies to oligodendrocytes promotes CNS remyelination.

Asakura K, Miller DJ, Pease LR, Rodriguez M

Department of Neurology, Mayo Clinic and Foundation, Rochester, Minnesota 55905, USA.

We previously identified the remyelinating activity of a natural IgMkappa oligodendrocyte-reactive autoantibody (SCH94.03), using a virus-induced murine model of multiple sclerosis. We now describe a second mouse IgMkappa monoclonal antibody (mAb) (SCH79.08) raised against normal mouse spinal cord homogenate, which reacts with myelin basic protein and also promotes remyelination. Because these two mAbs recognize different oligodendrocyte antigens, several previously identified oligodendrocyte-reactive IgMkappa mAbs (O1, O4, A2B5, and HNK-1), each with distinct antigen specificities, were evaluated and found to promote remyelination. In contrast, IgMkappa mAbs that did not bind to oligodendrocytes showed no remyelination. One of these, CH12 IgMkappa mAb, which shares variable region cDNA sequences with SCH94.03 except for amino acid differences in the complementarity-determining region 3 in both heavy and light chains, did not bind to oligodendrocytes and did not promote remyelination. The fact that multiple oligodendrocyte-reactive antibodies with distinct antigen reactivities induce remyelination argues against direct activation by a unique cell surface receptor. These findings are most consistent with the hypothesis that the binding of mAbs to oligodendrocytes in the lesions induces myelin repair via indirect immune effector mechanisms initiated by the mu-chain. Importantly, these studies indicate that oligodendrocyte-reactive natural autoantibodies may provide a powerful and novel therapeutic means to induce remyelination in multiple sclerosis patients.

PMID: 9742140, UI: 98414541

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## Neurotransmitter receptor activation triggers p27(Kip1) and p21(CIP1) accumulation and G1 cell cycle arrest in oligodendrocyte progenitors.

Ghiani CA, Eisen AM, Yuan X, DePinho RA, McBain CJ, Gallo V

Laboratory of Cellular and Molecular Neurophysiology, NICHD, NIH, Bethesda, MD 20892-4495, USA.

We examined the pathways that link neurotransmitter receptor activation and cell cycle arrest in oligodendrocyte progenitors. We had previously demonstrated that glutamate receptor activation inhibits oligodendrocyte progenitor proliferation and lineage progression. Here, using purified oligodendrocyte progenitors and cerebellar slice cultures, we show that norepinephrine and the beta-adrenergic receptor agonist isoproterenol also inhibited the proliferation, but in contrast to glutamate, isoproterenol stimulated progenitor lineage progression, as determined by O4 and O1 antibody staining. This antiproliferative effect was specifically attributable to a beta-adrenoceptor-mediated increase in cyclic adenosine monophosphate, since analogs of this cyclic nucleotide mimicked the effects of isoproterenol on oligodendrocyte progenitor proliferation, while alpha-adrenoceptor agonists were ineffective. Despite the opposite effects on lineage progression, both isoproterenol and the glutamate receptor agonist kainate caused accumulation of the cyclin-dependent kinase inhibitors p27(Kip1) and p21(CIP1), and G1 arrest. Studies with oligodendrocyte progenitor cells from INK4a<sup>-/-</sup> mice indicated that the G1 cyclin kinase inhibitor p16(INK4a) as well as p19(ARF) were not required for agonist-stimulated proliferation arrest. Our results demonstrate that beta-adrenergic and glutamatergic receptor activation inhibit oligodendrocyte progenitor proliferation through a mechanism that may involve p27(Kip1) and p21(CIP1); but while neurotransmitter-induced accumulation of p27(Kip1) is associated with cell cycle arrest, it does not by itself promote oligodendrocyte progenitor differentiation.

PMID: 9927607, UI: 99128196

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## Development of O4+/O1- immunopanned pro-oligodendroglia in vitro.

Ingraham CA, Rising LJ, Morihisa JM

Department of Psychiatry, Albany Medical College, NY 12208, USA. christine\_ingraham@ccgateway.amc.edu

In this study, O4+/O1- pro-oligodendroglia isolated by immunopanning from cerebral hemispheres of P3-P5 rats were evaluated during their maturation in culture. Immunopanning yielded  $3-4 \times 10^5$  cells/cerebrum, with 98% O4+ and 6% O1+. There was heterogeneity in the morphologies of immunopanned cells ranging from simple bipolar cells to more complex multipolar cells. As a first step in determining potential differentiative responses of mature oligodendroglia, we examined glial fibrillary acidic protein (GFAP) expression in response to fetal bovine serum (FBS) by cultures established from O4+/O1- immunopanned cells grown for 1, 14, or 21 days, exposed to 20% FBS for 6-7 days and fixed and immunostained on days 7, 21 or 28 in culture (DIC). When immunopanned cells were exposed to FBS following 1 day in serum-free medium, 88% expressed GFAP and when immunopanned cells were cultured for 14 days prior to FBS exposure, 78% expressed GFAP. By contrast, when cells were cultured for 21 days prior to FBS exposure (when a majority of the cells expressed O1 and myelin basic protein (MBP)), only 19% of the cells expressed GFAP ( $p < 0.001$ ). Cells that were O4+/GFAP- even in the presence of FBS often exhibited a mature oligodendroglial morphology. Among immunopanned cells that responded to FBS by expression of GFAP, both process-bearing (similar to type 2 astroglia) and flattened, polygonal (similar to type 1 astroglia) GFAP+ cells were observed. These results confirm the utility of immunopanning for the isolation of pro-oligodendroglia and demonstrate that oligodendroglia that develop in vitro from O4+/O1- immunopanned cells become resistant to GFAP induction by FBS.

PMID: 9974161, UI: 99139741

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